REMARKS

Claims 109-130 are pending. Claims 112-115, 117, and 128-130 have been withdrawn. Claim 118 is cancelled. Claims 109 and 127 have been amended. Support for the amendments may be found in Example 2 of the specification.

I. Summary of the Invention

Amended claim 109 encompasses a biosensor that comprises two nucleic acid constructs, represented by R1-R2-R3-R4 and R5-R6-R7-R8. Generally speaking, when each construct of the biosensor binds to the same target, the biosensor produces a detectable signal. To accomplish this, R1 is an epitope binding agent that binds to a first epitope on a target molecule, and R5 is an epitope binding agent that binds to a second epitope on the target molecule. R2 is a *non-nucleic acid* flexible linker attaching R1 to R3, while R6 is a *non-nucleic acid* flexible linker attaching R5 to R7. R3 is a nucleotide sequence that is complementary to R7. R3 and R7 have a free energy for association from *about 5.5 kcal/mole to about 8.0 kcal/mole* at a temperature from about 21° C to about 40° C and at a salt concentration from about 1 mM to about 100 mM. R4 and R8 together comprise a detection means such that when R3 and R7 associate a detectable signal is produced.

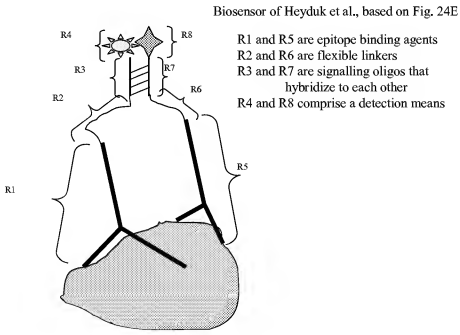
Typically, when R1 and R5 bind to the same target, the non-nucleic acid linkers (i.e. R2 and R6) are of a length and flexibility that allows R3 and R7 to stably hybridize. This brings R4 and R8 into close proximity, producing a detectable signal. The hybridization of R3 and R7 is critical to the sensitivity of the biosensor, and is dependent, in part, on the free energy of association between R3 and R7 and the non-nucleic acid linkers R2 and R6. In view of the above, it is respectfully submitted that the prior art does not disclose, teach, or suggest the currently claimed biosensors of claim 109. In particular, nowhere does the cited art disclose or suggest non-nucleic acid linkers or a ΔG of between about 5.5 kcal/mol and about 8.0 kcal/mol.

II. §102 Rejection

Reconsideration is requested of the rejection of claims 109-111, 116, 118-122 and 124-127 under 35 USC §102(b) in view of published US Patent Application No. 2002/0051986 to Baez et al, as evidenced by SantaLucia (PNAS, 1998, 95, 1460-1465).

Amended claim 109 encompasses a biosensor that comprises two nucleic acid constructs, represented by R1-R2-R3-R4 and R5-R6-R7-R8. R2 is a *non-nucleic acid* flexible linker attaching R1 to R3, while R6 is a *non-nucleic acid* flexible linker attaching R5 to R7. R3 is a nucleotide sequence that is complementary to R7. R3 and R7 have a free energy for association from about 5.5 kcal/mole to about 8.0 kcal/mole at a temperature from about 21° C to about 40° C and at a salt concentration from about 1 mM to about 100 mM. An illustration of a biosensor of claim 109 may be found in Diagram 1A below.

Diagram 1A:

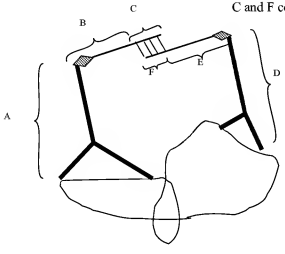


The Baez et al. application discloses a nucleic acid based reporter that may be used to detect an analyte. The Baez reporter comprises two constructs. Each construct comprises an antibody (A and D in Diagram 1B) and a nucleic acid reporter (B and C together, and E and F together, in Diagram 1B). When each antibody binds to the target analyte, the two nucleic acid reporters are brought into close proximity, and a signal may be produced. Unlike the biosensor of amended claim 109, however, the nucleic acid reporter (B and C together, and E and F together, in Diagram 1B) of the Baez biosensor is ***comprised solely of nucleic acid***.

Diagram 1B:

Biosensor of Baez et al, based on Fig. 1 and 3

According to the Office,
A and D correspond to R1 and R5
B and E correspond to R2 and R6
C and F correspond to R3 and R7



A claim is anticipated only if each and every element as set forth in the claim is described in a single prior art reference.[1] The Baez application sensor, however, fails to disclose at least two elements of the biosensor of claim 109: a non-DNA linker (i.e. R2 or R6), and a complementary sequence with a free energy between about 5.5 kcal/mol and about 8.0 kcal/mol. Each is discussed in more detail below.

[1] *Verdegaal Bros. v. Union Oil Co. of Calif.*, 2 U.S.P.Q 2d 1051, 1053 (Fed. Cir. 1987).

According to the Office, R2 and R6 (the flexible linkers of the Heyduk biosensor) correspond to a portion of the nucleic acid reporter of Baez et al. Specifically, the Office states: "[t]he linker comprising [the] *nucleic acid region not complementary to the nucleic acids* [i.e. B and E of Diagram 1B] are the flexible linker R2 … and R6," whereas, "[t]he *nucleic acid region comprising the complementary region* [i.e. C and F of attached Diagram 1B] are the 'R3' and 'R7' of the instant claim."[2]

(a) non-DNA linker (i.e. R2 and R6)

Claim 109 limits R2 and R6 to ***non-nucleic acid linkers***. The Baez application only discloses a B and E (according to Diagram 1B, i.e. R2 and R6 according to the Office), comprised of nucleic acid. Hence, the Baez application does not anticipate claim 109 because its linker is nucleic acid, which is expressly excluded by the limitations of amended claim 109.

(b) free energy

Additionally, as detailed above, claim 109 states that the free energy of association for R3 and R7 must be between about 5.5 kcal/mol and about 8.0 kcal/mole. The Baez application, in contrast, does not teach a free energy of association for C and F. The Baez application actually provides only one complementary sequence, namely 5'-CGCCCGA-3', which the Office correlates to R3 or R7. This sequence, however, does ***not have*** a free energy of association between about 5.5 kcal/mol and about 8.0 kcal/mol.

This is evidenced by the attached declaration of Dr. Tomasz Heyduk ("the Heyduk declaration"), where Dr. Heyduk states:

> it is my considered belief that the signaling oligonucleotide from the Baez application (CGC CCG A; sequence from oligonucleotide constructs T68 and T66, Table 1 of Baez et al. patent; C and F of Diagram 1) does not have a free

[2] See the Office Action mailed July 2, 2008, at page 5.

energy of association within the limits of claim 109 (e.g. within about 5.5 kcal/mol and about 8.0 kcal/mol).[3]

To support his conclusion, Dr. Heyduk calculated the free energies of hybridization using "the program Hyther from the web site of Dr. John SantaLucia Jr. (http://ozone3.chem.wayne.edu/)."[4] This is the same Dr. John SantaLucia that "authored the paper the Office has cited for calculating free energy values,"[5] namely SantaLucia (PNAS, 1998, 95, 1460-1465). Importantly, the website allows calculations at various salt concentrations.

Using the website of Dr. SantaLucia, Dr. Heyduk has determined that "the ΔG for the Baez sequence (CGC CCG A) in 0.1 M salt at 20°C is **10.43 kcal/mole** (11.92 at 1 M salt)."[6] Hence, the Baez application **does not** teach a complementary nucleic acid sequence with a free energy of association between about 5.5 kcal/mol and about 8.0 kcal/mol. Thus, the Baez application cannot anticipate claim 109 of the present application.

The Office calculated the free energy of the Baez complementary sequence to be 6.91 kcal/mol using the SantaLucia PNAS reference. This value is incorrect, however. The Office's calculations are based only on the value for initiation of a sequence that terminates in GC (0.98) or AT (1.03), not the ΔG° values for each base pair, as exemplified in Figure 1 of the SantaLucia application.

Similar to claim 109, claims 110, 111, 116, 119 –122, and 124 -126 each depend from claim 109, and therefore necessarily incorporate each limitation of claim 109. Consequently, the Baez application cannot anticipate claims 110, 111, 116, 119 –122, and 124 -126 for the same reasons as detailed above with respect to claim 109.

---

[3] See point 2(b) of the Heyduk declaration.
[4] See point 2(b)(i) of the Heyduk declaration.
[5] *Id.*
[6] See point 2(b)(i)(1) of the Heyduk declaration.

Claim 127, analogous to claim 109, requires that R2 and R6 are not comprised of nucleic acid, and that R3 and R7 have a free energy of association between about 5.5 kcal/mol and 8.0 kcal/mol. The Baez application, which only discloses linkers of nucleic acids and does not disclose a complementary nucleic acid region with a free energy between about 5.5 kcal/mol and about 8.0 kcal/mol, does not, therefore, anticipate claim 127 for the same reasons detailed above with respect to claim 109.

Consequently, Applicant requests withdrawal of the rejection of claims 109-111, 116, 118-122 and 124-127 under §102b in view of Baez et al.

III. §103 Rejection

Reconsideration is requested of the rejection of claims 109 and 123 under 35 USC §103(a) in view of Baez et al, SantaLucia, and Zaplinsky.

As discussed above, claim 109 encompasses a biosensor comprising two different constructs. Each construct is comprised of an epitope binding agent that recognizes different epitopes of the same target. When the epitope binding agents bind to the target, the complementary nucleic acid sequences, R3 and R7 are brought close together and stably hybridize to each other. The hybridization brings two different labels into close proximity, producing a detectable signal. It is important that R3 and R7 have an appropriate free energy of association, so that they ***do not*** hybridize ***unless*** the epitope binding agents are bound to the target, but ***do*** hybridize when the epitope binding agents ***bind a target***. As such, claim 109 requires that R3 and R7 have a free energy of association between about 5.5 kcal/mol and about 8.0 kcal/mol. *None* of the references cited by the Office disclose a biosensor with a free energy of association between about 5.5 kcal/mol and about 8.0 kcal/mol, which the Applicants have unexpectedly shown to be critical to the biosensor of claim 109. This free energy range ensures, in part, that the signaling oligonucleotides (i.e. R3 and R7) ***do not*** hybridize ***unless*** the epitope binding agents are bound to the target, but ***do*** hybridize when the epitope binding agents ***bind a target***. Free energies outside of this range do not

strike this delicate balance, resulting in little to no signal, as indicated by the data detailed in Diagram 2.

(a) a prima facie case of obviousness has not been established in view of the cited art

Three criteria must be present to establish a prima facie case of obviousness.[7] First, the prior art reference must teach or suggest all the claim limitations. Second, there must be some suggestion or motivation in the knowledge generally available to one of ordinary skill in the art to modify the reference. Third, there must be a reasonable expectation of success.[8] Not one of these three criteria is satisfied by the combination of the Baez application, the SantaLucia reference, and the Zalipsky reference.

As discussed above, the Baez application discloses a sensor, but the Baez sensor does not disclose non-nucleic acid linkers, and does not teach a complementary nucleic acid sequence with a free energy of association between about 5.5 kcal/mol and 8.0 kcal/mol.

As stated by the Office, the SantaLucia reference was cited solely for the purpose of showing how to calculate free energy of association.

The Zalipsky reference discloses polyethyleneglycol (PEG) as a conjugate for biologically active molecules. By attaching PEG to the biologically active molecule, the molecule is stabilized. The Zilpinsky reference does not disclose using polyethyleneglycol as a ***linker*** between an epitope binding agent and a complementary nucleic acid in a biosensor. Nor does it disclose a biosensor with complementary nucleic acid sequences that have a free energy of association between about 5.5 kcal/mol and 8.0 kcal/mol.

In summary, *not one of the cited references, whether taken together or in combination, disclose a biosensor with non-nucleic acid linkers and complementary nucleic acid sequences that have a free energy of association*

---

[7] MPEP §2143
[8] *Id.*

between about 5.5 kcal/mol and 8.0 kcal/mol. In particular, not one of the references cited by the Office discusses the free energy of association for complementary nucleic acids. Because there is no mention of the free energy limitation in the cited art, the cited art doesn't provide a motivation to modify the references. Hence, Applicants respectfully submit that claim 109 is patentable over the cited art. Similarly, claim 123 depends from claim 109, and therefore, is patentable for the same reasons as detailed above with respect to claim 109.

(b) indicia of non-obviousness

Even assuming, *arguendo*, that a prima facie case of obviousness has been established in view of the prior art, this case can be rebutted by showing that the claimed biosensor achieves unexpected results. Dr. Heyduk has stated that:

> the combination of non-DNA flexible linkers with signaling oligonucleotides that have a free energy of association between about 5.5 kcal/mol and about 8.0 kcal/mol in a biosensor of claim 109 of the '107 application produces the unexpected benefit of significantly increased biosensor sensitivity.[9]

Dr. Heyduk's statement is based, in part, "on the data in attached Diagram 2. This data was from an experiment that compared the response of two different biosensors specific for C-peptide. The two separate biosensors were combined with their target, namely C-peptide, and the resulting FRET signal was measured over time."[10]

The first biosensor, based on a biosensor of claim 109, "utilized signaling oligonucleotides [i.e. R3 and R7 of Diagram 1A] that have a free energy of association between about 5.5 kcal/mol and about 8.0 kcal/mol."[11,12] Stated

---

[9] See point 2(a) of the Heyduk declaration.
[10] See point 2(a)(i) of the Heyduk declaration.
[11] See point 2(a)(i)(1) of the Heyduk declaration.

another way, this biosensor used signaling oligonucleotides that have a free energy for association within the limitations of claim 109. This biosensor produced a signal more than 7 times above baseline.

"The second biosensor used the overlap sequence described in the Baez et al. patent (CGC CCG A; sequence from oligonucleotide constructs T68 and T66, Table 1 of Baez et al. patent; C and F of Diagram 1), and had a free energy of association higher than 8.0 kcal/mol."[13] Stated another way, the Baez sensor used signaling oligonucleotides that have a free energy of association outside the limitations of claim 109. This resulted in "***essentially no signal***,"[14] as indicated in Diagram 2. This was despite the fact that Dr. Heyduk "attached the Baez sequence to the antibody via a non-DNA linker" known to "facilitate FRET signaling."[15]

Hence, using biosensors comprised of the *same* antibody (i.e. R1 and R5), the *same* linker (i.e. R2 and R6), and the *same* detection means (i.e. R4 and R8), but two different signaling oligos, with free energies of association either within the limitations of claim 109 or outside the limitations (the Baez sensor), resulted in a difference in signal specificity of more than 7 fold. No reference cited by the Office discloses the importance of the free energy of association of the signaling oligonucleotides in a biosensor of claim 109. Moreover, no reference cited by the Office combines the free energy limitation with the limitation of a non-nucleic acid linker. Consequently, the biosensor of claim 109, which is limited to signaling oligonucleotides that have a free energy range of

---

[12] Point 2(c) of the Heyduk declaration states: "It is also my considered belief, that using the SantaLucia website, the signaling oligonucleotide sequence ATG AGC used in the experiment … does have a free energy of association between about 5.5 kcal/mol and about 8.0 kcal/mol…the ΔG for the sequence used in the experiment above (ATG AGC) at 0.1 M salt and 20°C is 5.76 kcal/mole (7 kcal/mole at 1 M salt), which is within the boundaries of claim 109 (e.g. between about 5.5 kcal/mol and about 8.0 kcal/mol).

[13] See point 2(a)(i)(2) of the Heyduk declaration.

[14] See point 2(a)(iii) of the Heyduk declaration.

[15] *Id*.

about 5.5kcal.mol to about 8kcal/mol, and a non-nucleic acid linker, is not obvious in light of the art cited by the Office.

In view of the foregoing, the Applicants respectfully request withdrawal of the obviousness rejection of claim 109 and 123.

III. Double Patenting Rejections

Claims 109-111, 116, and 119-127are rejected for provisional nonstatutory obviousness-type double patenting in view of claims 1-11 of copending application no. 11/836,339 and claims 1-8 of copending application no. 11/836,333. Because the '339 and '333 applications have not issued as patents, as the Office correctly notes, the double patenting rejection is a provisional rejection. As such, the applicants will address the substantive merit of the double patenting rejection if and when either of the '339 or '332 applications issue as patents.

CONCLUSION

In light of the foregoing, applicants request entry of the claim amendments, withdrawal of the claim rejections, and solicit an allowance of the claims. The Examiner is invited to contact the undersigned attorney should any issues remain unresolved.

Respectfully submitted,

Polsinelli Shalton Flanigan Suelthaus PC

Date: December 31, 2008

By: /Rebecca C. Riley-Vargas/
Rebecca C. Riley-Vargas, Reg. No. 60,046
110 South Fourth Street, Suite 1100
St. Louis, MO 63102
Phone – 314-889-8000
Fax – 314-231-1776
Attorney